NEW TECHNIQUES TO INVESTIGATE THE ONTOGENY OF SCALPELLID BARNACLES, AND THEIR PHYLOGENETIC IMPLICATIONS

Scalpellid barnacles (Thoracica: Lepadomorpha: Scalpellidae) are stalked, with a capitulum bearing more than five calcified or partly calcified plates. They are poorly known and have been rare in museum collections. Species descriptions are often based on one or few specimens. It is often difficult to assign species to many of the proposed scalpellid genera. This paper describes techniques being developed to provide comprehensive descriptions of recent rich Australian collections. As some species are known by only a few individuals, emphasis has been placed on techniques which minimise trauma to the specimen. Conchological techniques, described as destructive, semi-destructive and non-destructive, are used to examine the calcareous capitular plates, since the arrangement of these is of systematic, ontogenetic and phylogenetic significance. Consideration is also given to staining techniques, since descriptions of morphological details of trophi, cirri and complemental or dwarf males (if present) are lacking for many species.

Conchological Techniques

Destructive techniques. These are the conventional dissection techniques. In some scalpellid species the capitular plates are covered by or embedded in a thick, often opaque membrane, and removal of the membrane and/or dissection of the plates is necessary. These destructive techniques may be acceptable if several specimens are available. They are not suitable if only a few specimens exist, or for ontogenetic studies. The inherent possibility exists that plates may be lost inadvertently during dissection and the possibility of damage to fragile plates is also very real.

Semi-destructive techniques. A. Bisection of the specimen, followed by back-lighting, provides an accurate representation of the relative positions of the capitular plates. The success of this technique depends on the relative thickness of the capitular plates, their proximity to one another, and the thickness of the investing membrane.

B. Cleared (transparent) and stained specimens are widely used for vertebrate osteological studies. Preparation of such specimens involves tissue maceration in alkaline solutions (KOH or NaOH), or tissue digestion by proteolytic enzymes (e.g. trypsin). Bones are then stained for maximum definition and visibility.

Thoracicans exhibit shells composed of $CaCO_3$, mainly in the form of calcite, with little phosphate or organic matter. The calcareous portion of the thoracican shell appears during metamorphosis of the cyprid larva into the young barnacle. Vertebrate clearing and staining techniques were applied, with modifications, to scalpellid specimens. Fresh and newly preserved material was successfully cleared used KOH, but the technique can be unpredictable and difficulties arose with specimens stored in various preservatives for any length of time. The proteolytic enzyme digestion method was more successful. It was easier to prepare small, delicate specimens, and capitular plates were not so easily damaged, distorted or lost. It was also easier to clear old material, although results were not always predictable. However, material properly preserved prior to enzyme digestion generally yielded good transparent study specimens. Enzyme digestion is less harmful to the specimens than alkaline maceration. Maximum satisfactory enzyme activity occurs at pH 7.5 or above.

For all except very small specimens we removed the prosoma before processing because this enables the most rapid enzyme digestion to occur and minimises injury to the capitulum. Difficulties encountered using the enzyme technique are: (i) determination of the length of time for tissue digestion, as specimens will fall apart and/or disintegrate if over-exposed; and (ii) the length of the total process (up to 2 months in enzyme solution). The major advantage of the technique is a permanent, three-dimensional record of the capitular plate architecture.

Non-destructive techniques. Scalpellid specimens were X-rayed (another vertebrate osteological technique) with the assumption that the calcareous capitular plates would be radio-opaque. The technique is quick, economic and easy to adapt to a variety of sizes of specimens. A permanent record is obtained of the relative positions of the plates. The technique is excellent for both ontological and comparative studies. An advantage of the process is that the whole animal may be kept intact, which is important when only one or a few specimens are known. However, we have found it preferable to use bisected specimens to prevent 'shadowing' of paired plates on the X-ray image, which can sometimes hinder conchological interpretation.

Soft-part Morphology

We have adapted methods previously employed by cirripede workers. A variety of biological stains were used to examine external and internal morphological details (fast green, methyl blue, lignin pink, chlorazol black, solophenyl blue). We obtained excellent results with lignin pink and solophenyl blue. Suitably stained material was mounted in corn syrup diluted 50:50 with distilled water.

Conclusion

In the specimens studied consistent morphological and conchological differences are recognizable between the major subfamilies of the Scalpellidae (*sensu* Zevina, 1981). At the supra-specific level there appear to be few gross differences in appendages between species but differences in the form of the capitular plates are demonstrable. Criteria for the presently proposed genera need to be more critically defined, especially in the Arcoscalpellinae, with due regard to ontogenetic variations in morphology and body anatomy, complemental and dwarf males anatomy, as well as geographic and bathymetric distributions. Much more sustained, deep-water collecting needs to be done, not only in Australia but elsewhere, before a proper understanding of taxonomic variation in the Scalpellidae can be reached. Only then will their phylogenetic evolution be elucidated.

Literature Cited

Zevina, G.B. 1981. Cirriped crustaceans of the Suborder Lepadomorpha (Cirripedia, Thoracica) of the Pacific Ocean. Part 1. Family Scalpellidae. Opredeliteli Faune SSSR 127: 1–398. [in Russian].

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